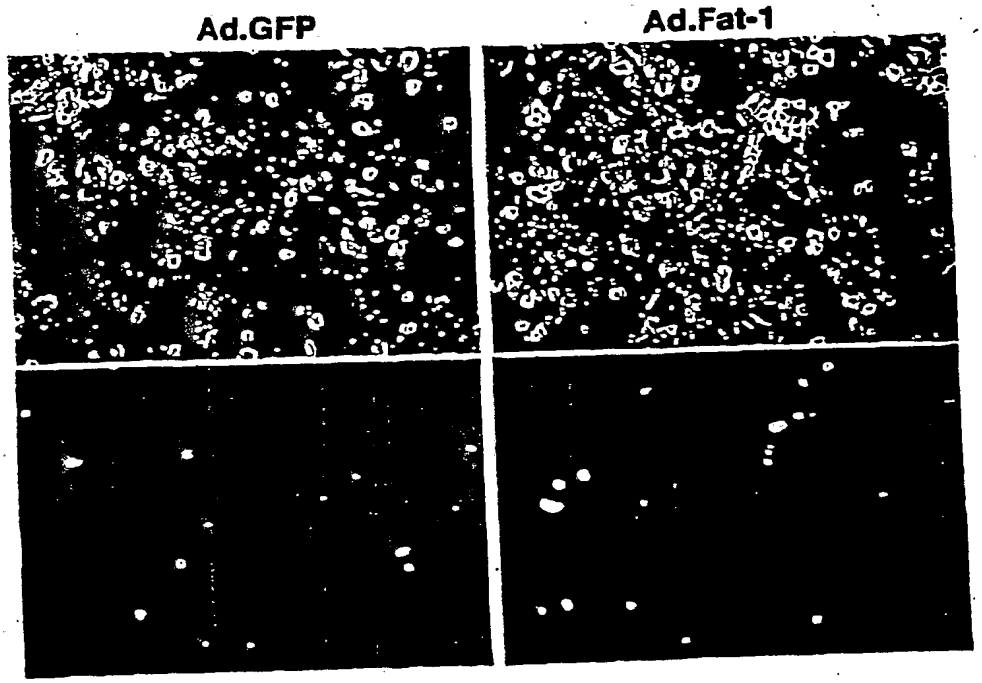


Fig. 1

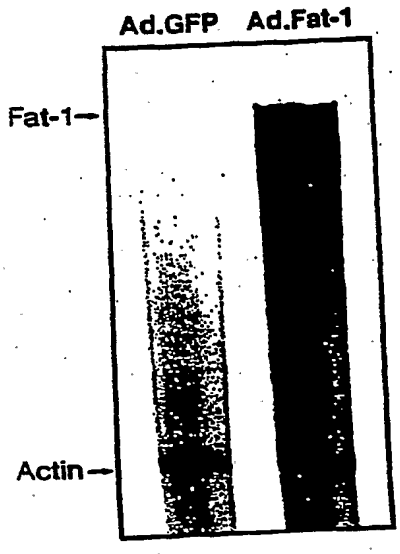


Fig. 2

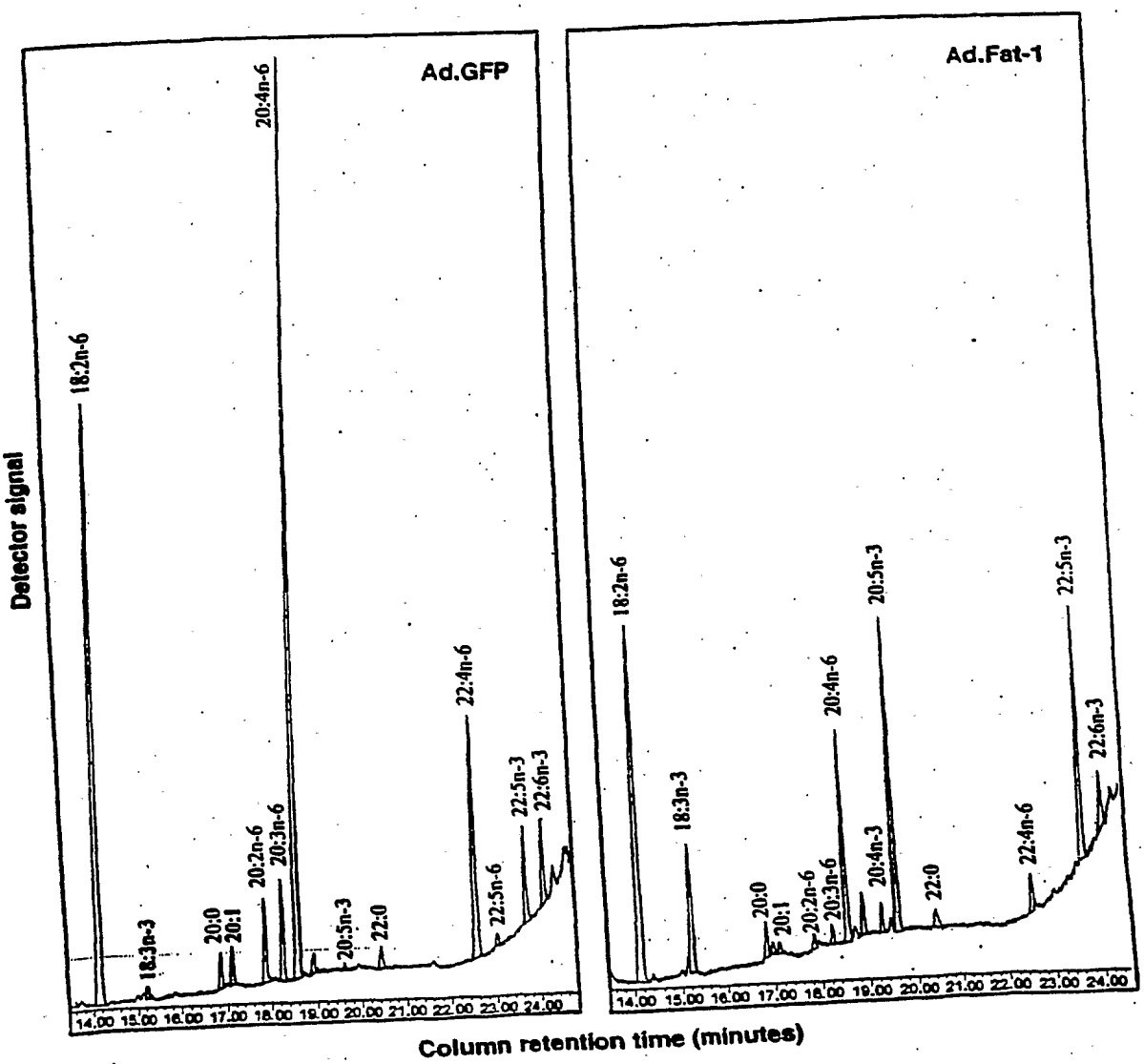

Ad.GFP
Ad.Fat-1
Detector signal
18:2n-6
18:3n-3
20:0
20:1
20:2n-6
20:4n-6
20:3n-6
20:5n-3
22:0
22:4n-6
22:5n-6
22:5n-3
22:6n-3
14.00 15.00 16.00 17.00 18.00 19.00 20.00 21.00 22.00 23.00 24.00
Column retention time (minutes)

Fig. 3

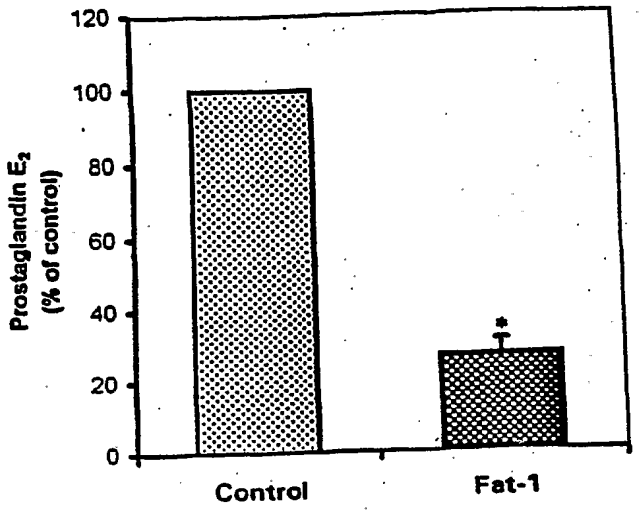

120
100
80
60
40
20
0
Prostaglandin E_2 (% of control)
*
Control
Fat-1

Fig. 4

Mol % of total Fatty acids	Control	Fat-1
n-6 Polyunsaturates		
18:2n-6	14.2[a]	9.2[b]
20:2n-6	1.2[a]	0.3[b]
20:3n-6	1.6[a]	0.4[b]
20:4n-6	15.2[a]	4.1[b]
22:4n-6	4.4[a]	1.0[b]
22:5n-6	0.2[a]	0.0[b]
Total	36.8[a]	15.0[b]
n-3 Polyunsaturates		
18:3n-3	0.2[b]	3.6[a]
20:4n-3	0.0[b]	0.6[a]
20:5n-3	0.1[b]	6.1[a]
22:5n-3	1.2[b]	5.8[a]
22:6n-3	1.0[a]	1.3[a]
Total	2.5[b]	17.4[a]
n-6/n-3 Ratio	14.7[a]	0.9[b]

Values are means of four measurements. Values for each fatty acid with the same letter do not differ significantly ($P<0.01$) between control and fat-1.

Fig. 5

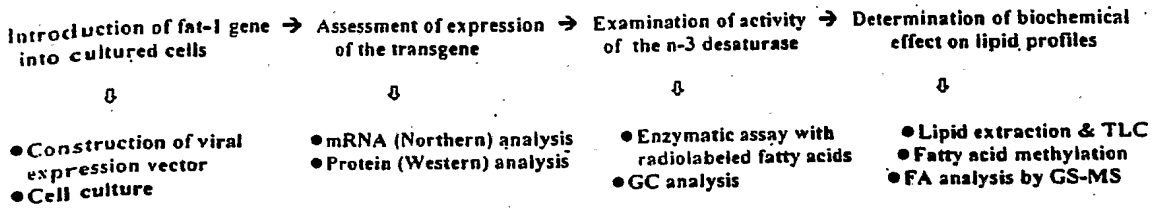

Introduction of fat-1 gene into cultured cells
Assessment of expression of the transgene
Examination of activity of the n-3 desaturase
Determination of biochemical effect on lipid profiles
Construction of viral expression vector
Cell culture
mRNA (Northern) analysis
Protein (Western) analysis
Enzymatic assay with radiolabeled fatty acids
GC analysis
Lipid extraction & TLC
Fatty acid methylation
FA analysis by GS-MS

Fig. 6

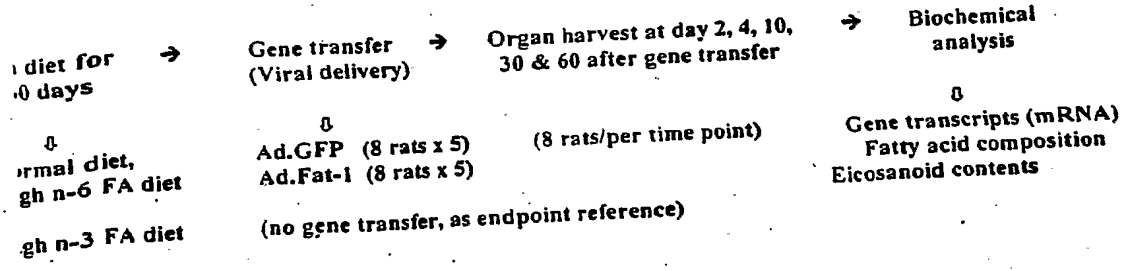

diet for
0 days
rmal diet,
gh n-6 FA diet
gh n-3 FA diet
Gene transfer
(Viral delivery)
Ad.GFP (8 rats x 5)
Ad.Fat-1 (8 rats x 5)
(no gene transfer, as endpoint reference)
Organ harvest at day 2, 4, 10, 30 & 60 after gene transfer
(8 rats/per time point)
Biochemical analysis
Gene transcripts (mRNA)
Fatty acid composition
Eicosanoid contents

Fig. 7

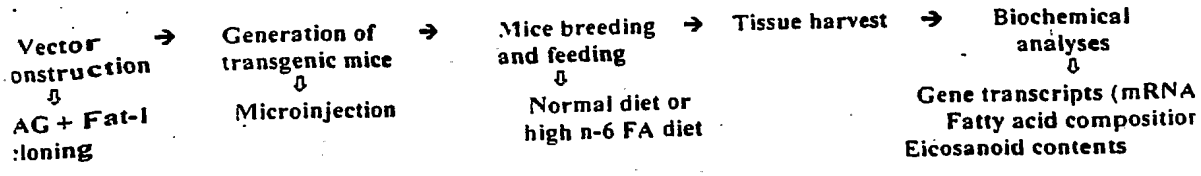

Vector onstruction
AG + Fat-1 :loning
Generation of transgenic mice
Microinjection
Mice breeding and feeding
Normal diet or high n-6 FA diet
Tissue harvest
Biochemical analyses
Gene transcripts (mRNA
Fatty acid compositior
Eicosanoid contents

Fig. 8

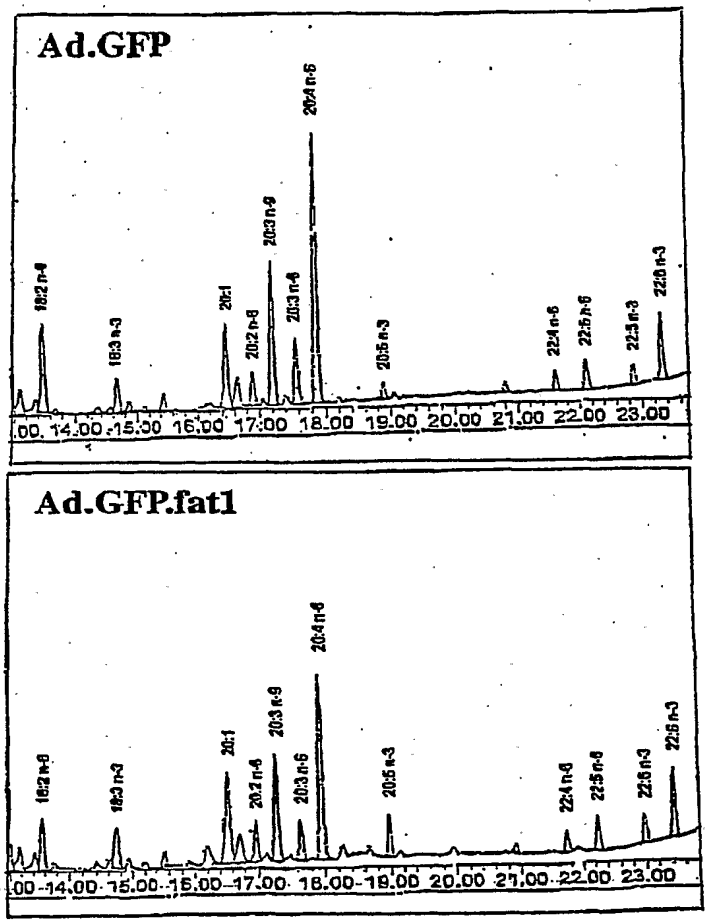

Ad.GFP
18:2 n-6
18:3 n-3
20:1
20:2 n-6
20:3 n-9
20:3 n-6
20:4 n-6
20:5 n-3
22:4 n-6
22:5 n-6
22:5 n-3
22:6 n-3
00 14.00 15.00 16.00 17:00 18.00 19.00 20.00 21.00 22.00 23.00
Ad.GFP.fat1
18:2 n-6
18:3 n-3
20:1
20:2 n-6
20:3 n-9
20:3 n-6
20:4 n-6
20:5 n-3
22:4 n-6
22:5 n-6
22:5 n-3
22:6 n-3
00 14.00 15.00 16.00 17.00 18.00 19.00 20.00 21.00 22.00 23.00

Fig. 9

PUFA composition of total cellular lipids from the control rat cortical and the transgenic cells expressing a *C. elegans fat-1* cDNA

Mol % of total fatty acids	Control	*fat-1*
n-6 Polyunsaturates		
18:2n-6	1.78	0.87
20:4n-6	7.21	4.23
22:4n-6	1.57	0.72
22:5n-6	1.68	0.72
Total	12.26	6.53
n-3 Polyunsaturates		
18:3n-3	0.34	0.86
20:5n-3	0.21	0.87
22:5n-3	0.29	0.81
22:6n-3	1.27	1.93
Total	2.11	4.48
n-6/n-3 Ratio	**6.44**	**1.67**

Values are means of four measurements. ($p<0.01$) between control and fat-1.

Fig. 10

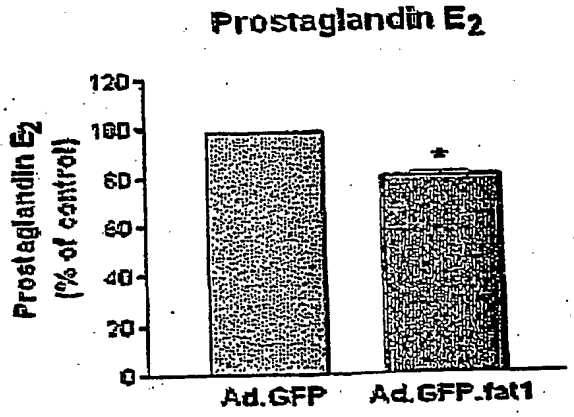

Prostaglandin E_2
Prostaglandin E_2 (% of control)
120
100
80
60
40
20
0
*
Ad.GFP
Ad.GFP.fat1

Fig. 11

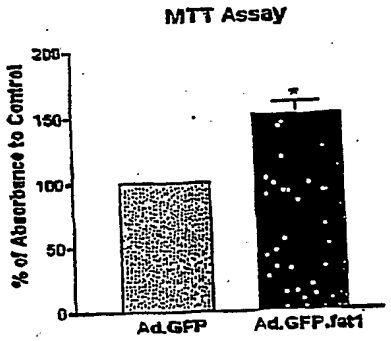

MTT Assay
% of Absorbance to Control
200
150
100
50
0
*
Ad.GFP
Ad.GFP.fat1

Fig. 12

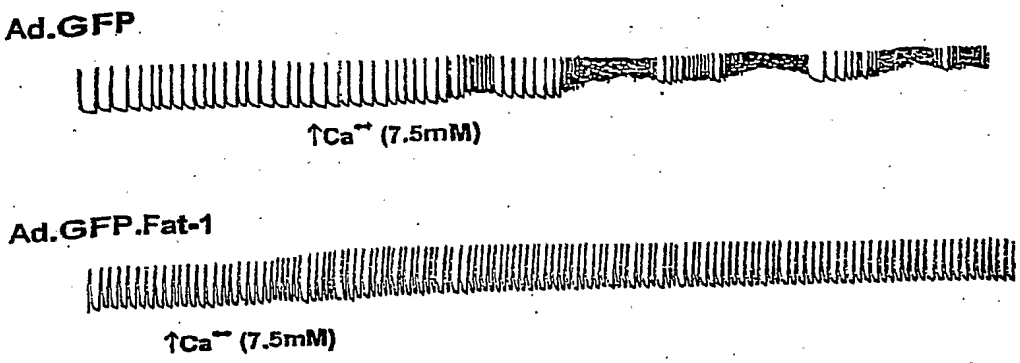


Fig. 13

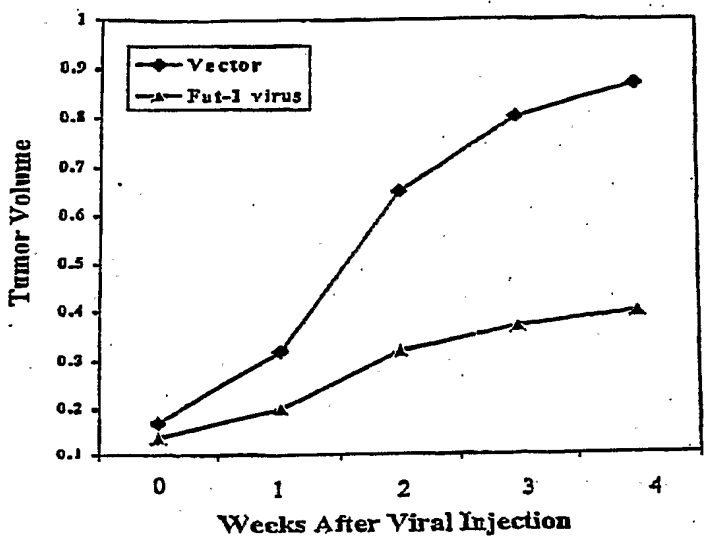

Vector
Fut-1 virus
Tumor Volume
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1
2
3
4
Weeks After Viral Injection

Fig. 14

PUFA composition of total cellular lipids from the control MCF-7 and the transgenic cells expressing a *C. elegans fat-1* cDNA

Mol % of total fatty acids	Control	Fat-1
n-6 Polyunsaturates		
18:2n-6	3.13[a]	1.51[b]
20:2n-6	0.23[a]	0.22[a]
20:3n-6	0.34[a]	0.16[b]
20:4n-6	6.30[a]	2.26[b]
22:4n-6	0.53[a]	0.33[b]
22:5n-6	0.27[a]	0.11[b]
Total	10.80[a]	4.59[b]
n-3 Polyunsaturates		
18:3n-3	0.0[b]	1.00[a]
20:4n-3	0.0[b]	0.10[a]
20:5n-3	0.0[b]	2.87[a]
22:5n-3	0.33[b]	1.47[a]
22:6n-3	0.60[a]	0.73[a]
Total	0.93[b]	6.17[b]
n-6/n-3 Ratio	11.61[a]	0.74[b]

Values are means of four measurements. Values for each fatty acid with the same letter do not differ significantly ($p<0.01$) between control and fat-1.

Fig. 15

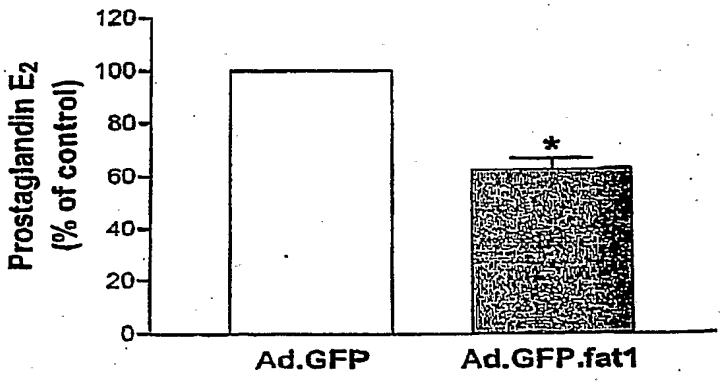

Prostaglandin E2
(% of control)
120
100
80
60
40
20
0
*
Ad.GFP
Ad.GFP.fat1

Fig. 16

```
CAAGTTTGAG GT                                                          --

ATG GTC GCT CAT TCC TCA GAA GGG TTA TCC GCC ACG GCT CCG GTC             57
Met Val Ala His Ser Ser Glu Gly Leu Ser Ala Thr Ala Pro Val
 5                  10                  15

ACC GGC GGA GAT GTT CTG GTT GAT GCT CGT GCA TCT CTT GAA GAA            102
Thr Gly Gly Asp Val Leu Val Asp Ala Arg Ala Ser Leu Glu Glu
            20                  25                  30

AAG GAG GCT CCA CGT GAT GTG AAT GCA AAC ACT AAA CAG GCC ACC            147
Lys Glu Ala Pro Arg Asp Val Asn Ala Asn Thr Lys Gln Ala Thr
        35                  40                  45

ACT GAA GAG CCA CGC ATC CAA TTA CCA ACT GTG GAT GCT TTC CGT            192
Thr Glu Glu Pro Arg Ile Gln Leu Pro Thr Val Asp Ala Phe Arg
    50                  55                  60

CGT GCA ATT CCA GCA CAC TGT TTC GAA AGA GAT CTC GTT AAA TCA            237
Arg Ala Ile Pro Ala His Cys Phe Glu Arg Asp Leu Val Lys Ser
65                  70                  75

ATC AGA TAT TTG GTG CAA GAC TTT GCG GCA CTC ACA ATT CTC TAC            282
Ile Arg Tyr Leu Val Gln Asp Phe Ala Ala Leu Thr Ile Leu Tyr
            80                  85                  90

TTT GCT CTT CCA GCT TTT GAG TAC TTT GGA TTG TTT GGT TAC TTG            327
Phe Ala Leu Pro Ala Phe Glu Tyr Phe Gly Leu Phe Gly Tyr Leu
        95                  100                 105

GTT TGG AAC ATT TTT ATG GGA GTT TTT GGA TTC GCG TTG TTC GTC            372
Val Trp Asn Ile Phe Met Gly Val Phe Gly Phe Ala Leu Phe Val
    110                 115                 120

GTT GGA CAC GAT TGT CTT CAT GGA TCA TTC TCT GAT AAT CAG AAT            417
Val Gly His Asp Cys Leu His Gly Ser Phe Ser Asp Asn Gln Asn
125                 130                 135

CTC AAT GAT TTC ATT GGA CAT ATC GCC TTC TCA CCA CTC TTC TCT            462
Leu Asn Asp Phe Ile Gly His Ile Ala Phe Ser Pro Leu Phe Ser
            140                 145                 150

CCA TAC TTC CCA TGG CAG AAA AGT CAC AAG CTT CAC CAT GCT TTC            507
Pro Tyr Phe Pro Trp Gln Lys Ser His Lys Leu His His Ala Phe
        155                 160                 165

ACC AAC CAC ATT GAC AAA GAT CAT GGA CAC GTG TGG ATT CAG GAT            552
Thr Asn His Ile Asp Lys Asp His Gly His Val Trp Ile Gln Asp
    170                 175                 180

AAG GAT TGG GAA GCA ATG CCA TCA TGG AAA AGA TGG TTC AAT CCA            597
Lys Asp Trp Glu Ala Met Pro Ser Trp Lys Arg Trp Phe Asn Pro
185                 190                 195

ATT CCA TTC TCT GGA TGG CTT AAA TGG TTC CCA GTG TAC ACT TTA            642
Ile Pro Phe Ser Gly Trp Leu Lys Trp Phe Pro Val Tyr Thr Leu
            200                 205                 210

TTC GGT TTC TGT GAT GGA TCT CAC TTC TGG CCA TAC TCT TCA CTT            687
```

Fig. 17A

```
Phe Gly Phe Cys Asp Gly Ser His Phe Trp Pro Tyr Ser Ser Leu
                215                 220                 225

TTT GTT CGT AAC TCT GAC CGT GTT CAA TGT GTA ATC TCT GGA ATC   732
Phe Val Arg Asn Ser Asp Arg Val Gln Cys Val Ile Ser Gly Ile
                230                 235                 240

TGT TGC TGT GTG TGT GCA TAT ATT GCT CTA ACA ATT GCT GGA TCA   777
Cys Cys Cys Val Cys Ala Tyr Ile Ala Leu Thr Ile Ala Gly Ser
                245                 250                 255

TAT TCC AAT TGG TTC TGG TAC TAT TGG GTT CCA CTT TCT TTC TTC   822
Tyr Ser Asn Trp Phe Trp Tyr Tyr Trp Val Pro Leu Ser Phe Phe
                260                 265                 270

GGA TTG ATG CTC GTC ATT GTT ACC TAT TTG CAA CAT GTC GAT GAT   867
Gly Leu Met Leu Val Ile Val Thr Tyr Leu Gln His Val Asp Asp
                275                 280                 285

GTC GCT GAG GTG TAC GAG GCT GAT GAA TGG AGC TTC GTC CGT GGA   912
Val Ala Glu Val Tyr Glu Ala Asp Glu Trp Ser Phe Val Arg Gly
                290                 295                 300

CAA ACC CAA ACC ATC GAT CGT TAC TAT GGA CTC GGA TTG GAC ACA   957
Gln Thr Gln Thr Ile Asp Arg Tyr Tyr Gly Leu Gly Leu Asp Thr
                305                 310                 315

ACG ATG CAC CAT ATC ACA GAC GGA CAC GTT GCC CAT CAC TTC TTC  1002
Thr Met His His Ile Thr Asp Gly His Val Ala His His Phe Phe
                320                 325                 330

AAC AAA ATC CCA CAT TAC CAT CTC ATC GAA GCA ACC GAA GGT GTC  1047
Asn Lys Ile Pro His Tyr His Leu Ile Glu Ala Thr Glu Gly Val
                335                 340                 345

AAA AAG GTC TTG GAG CCG TTG TCC GAC ACC CAA TAC GGG TAC AAA  1092
Lys Lys Val Leu Glu Pro Leu Ser Asp Thr Gln Tyr Gly Tyr Lys
                350                 355                 360

TCT CAA GTG AAC TAC GAT TTC TTT GCC CGT TTC CTG TGG TTC AAC  1137
Ser Gln Val Asn Tyr Asp Phe Phe Ala Arg Phe Leu Trp Phe Asn
                365                 370                 375

TAC AAG CTC GAC TAT CTC GTT CAC AAG ACC GCC GGA ATC ATG CAA  1182
Tyr Lys Leu Asp Tyr Leu Val His Lys Thr Ala Gly Ile Met Gln
                380                 385                 390

TTC CGA ACA ACT CTC GAG GAG AAG GCA AAG GCC AAG TAA          1221
Phe Arg Thr Thr Leu Glu Glu Lys Ala Lys Ala Lys
                395                 400

AAGAATATCC CGTGCCGTTC TAGAGTACAA CAACAACTTC TGCGTTTTCA       1271

CCGGTTTTGC TCTAATTGCA ATTTTTCTTT GTTCTATATA TATTTTTTTG       1321

CTTTTTAATT TTATTCTCTC TAAAAAACTT CTACTTTTCA GTGCGTTGAA       1371

TGCATAAAGC CATAACTCTT                                       1391
```

Fig. 17B

Optimized Tat-1 sequence:

```
1.    caa gtt tga ggt ATG gtc gct cat tcc AGC gaa ggg CtG tcc gcc acg gct ccg gtc acc
61.   ggc ggC gat gtG ctg gtG gat gcC cgt gca tct ctG gaG gaG aag gag gcC ccC cgC gaC
121.  gtg aat gca aac act aaa cag gcc acc act gaG gag ccC cgc atc caG tta ccC act gtg
181.  gat gcC ttc cgC cgC gca att ccC gca cac tgC ttc gaG agG gaC ctc gtG aaa tca atc
241.  agG tat Ctg gtg caG gac ttt gcg gca ctG aca att ctG tac ttt gcC ctt ccC gcC ttt
301.  gag tac ttt ggC Ctg ttt ggt tac Ctg gtG tgg aac att ttt atg ggC gtt ttt ggC ttc
361.  gcg Ctg ttc gtc gtt gga cac gaC tgt ctt caC ggC tca ttc tcC gat aat cag aat ctc
421.  aat gat ttc att gga cat atc gcc ttc AGC cca ctc ttc tct ccC tac ttc ccC tgg cag
481.  aaa agt cac aag ctG cac caC gcC ttc acc aac cac atC gac aaa gat cat gga cac gtg
541.  tgg atA cag gat aag gat tgg gaa gca atg ccC AGC tgg aaa aga tgg ttc aat ccT att
601.  ccT ttc tct ggC tgg ctG aaa tgg ttc ccT gtg tac act CtG ttc ggt ttc tgC gat gga
661.  tcC cac ttc tgg ccT tac tcC tca ctG ttt gtG cgC aac tct gaa cgC gtt caG tgt gta
721.  atc tct gga atc tgC tgc tgt gtg tgC gca tat att gct cta aca att gct gga AGC tat
781.  tcc aat tgg ttc tgg tac tat tgg gtt cca ctt tct ttc ttc ggC ttg atg ctc gtc att
841.  gtt acc tat Ctg caG caC gtc gaC gaC gtc gct gag gtg tac gag gct gat gaa tgg agc
901.  ttc gtc cgG gga caG acc caG acc atc gat cgt tac tat ggC ctc ggC ttg gac aca acg
961.  atg cac cat atc aca gac gga cac gtt gcc caC cac ttc ttc aac aaa atc cca cat tac
1021. cat ctc atc gaa gca acc gaa ggt gtc aaa aag gtc ttg gag ccg ttg tcc gac acc caa
1081. tac ggg tac aaa tct caG gtg aac tac gat ttc ttt gcc cgG ttc ctg tgg ttc aac tac
1141. aag ctc gac tat ctc gtt cac aag acc gcc gga atc atg caa ttc cga aca act ctc gag
1201. gag aag gca aag gcc aag tGa aag aat atc ccg tgc cgt tct aga gta caa caa caa ctt
1261. ctg cgt ttt cac cgg ttt tgc tct aat tgc aat ttt tct ttg ttc tat ata tat ttt ttt
1321. gct ttt taa ttt tat tct ctc taa aaa act tct act ttt cag tgc gtt gaa tgc ata aag
1381. cca taa ctc tt
```

Fig. 18

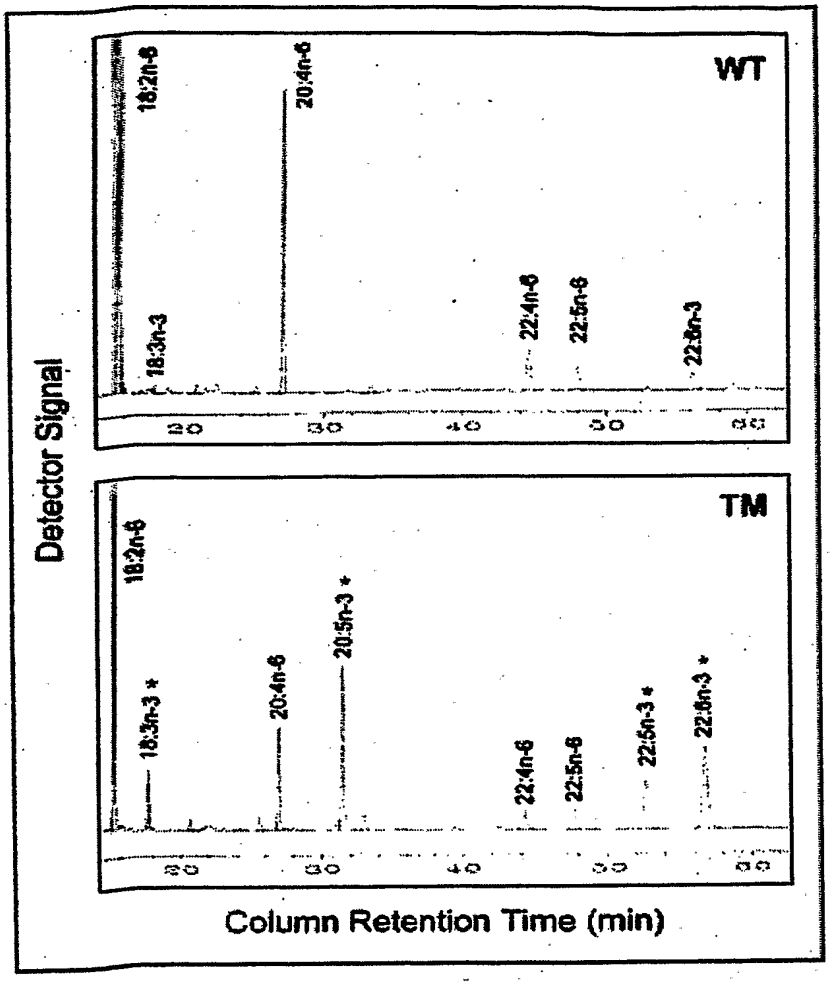

Detector Signal
WT
18:2n-6
20:4n-6
18:3n-3
22:4n-6
22:5n-6
22:6n-3
TM
18:2n-6
18:3n-3 *
20:4n-6
20:5n-3 *
22:4n-6
22:5n-6
22:5n-3 *
22:6n-3 *
Column Retention Time (min)

Fig. 19

Fig. 20

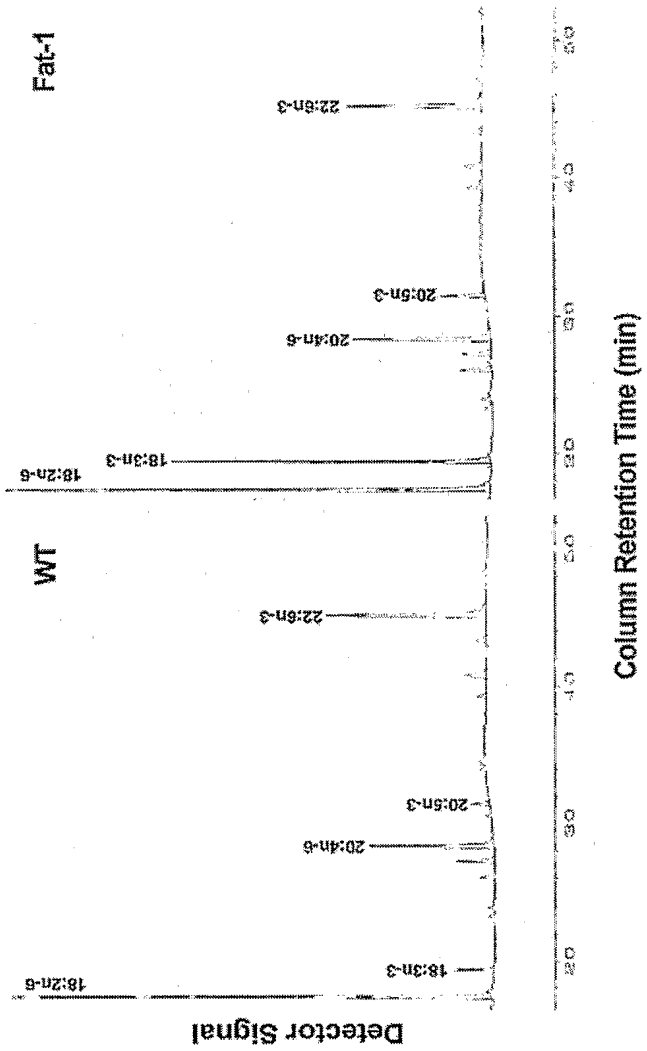
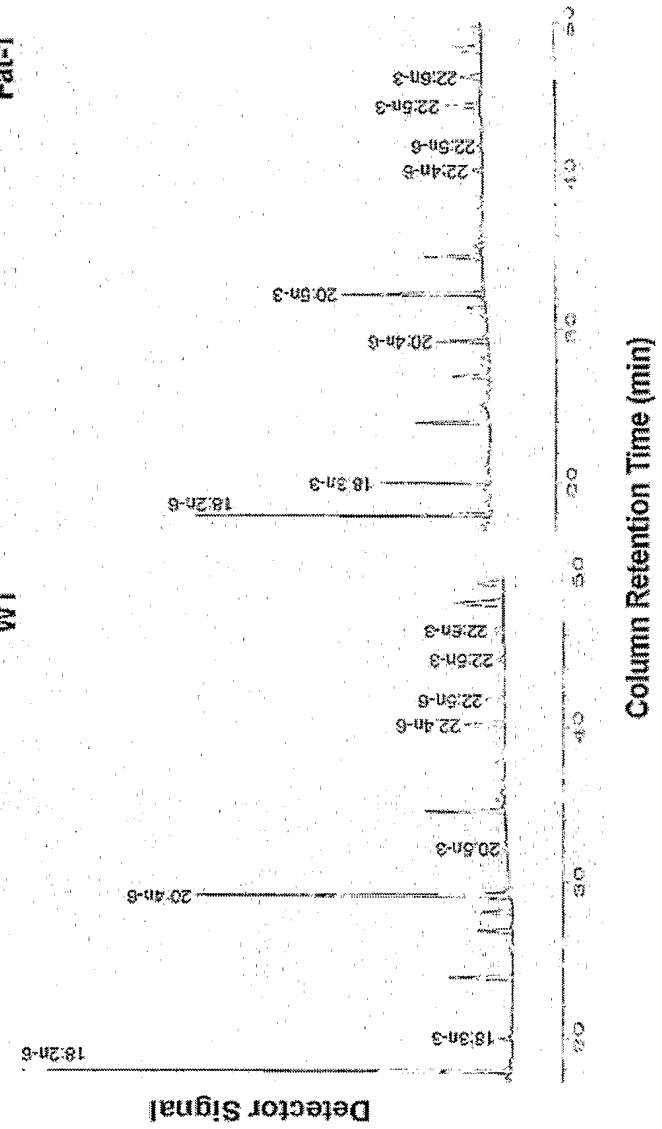

Fat-1
WT
18:2n-6
18:3n-3
20:4n-6
20:5n-3
22:6n-3
Detector Signal
Column Retention Time (min)

Fig. 21


Fat-1
WT
18:2n-6
18:3n-3
20:4n-6
20:5n-3
22:4n-6
22:5n-6
22:5n-3
Detector Signal
Column Retention Time (min)
0
10
20
30
40
50